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Case Report

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The Subcutaneous Tissue Reaction on Poly (L-lactide-co-caprolactone) based Threads

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Abstract

The thread-lifting procedure is gaining popularity all over the globe. It is a mini-invasive, rejuvenate procedure with a short downtime. Aesthetic practitioners use various medical devises and thread materials during thread lifting procedures, but thread degradation and tissue reaction on thread implants are currently poorly understood.

***Objective:** This article will describe tissue reaction and thread changes from implantation to full degradation.*

***Methods:** Tissue reaction on L-lactide-co-caprolactone threads was observed on 0, 4, 13, 26, 34, 52, 64, and 72 weeks after implantation in 21 rabbit models. In all groups, except the 26-week group, eight implants were placed in each model. Specifically, four Test Articles-Aptos Excellence Visage poly (L-lactide-co-caprolactone) threads were implanted on the right side in the cranial-caudal direction, and four control articles were implanted on the left side in the cranial-caudal direction.*

***Results:** Analysis of the local tissue reaction showed that the Test Article and the control article caused the same tissue reaction. However, compared with the control article, Test Article was associated with higher numbers of inflammatory cells on 13 and 34–72 weeks. At 72 weeks, the average area of Test Article had decreased by 41.1%.*

***Conclusion**: After subcutaneous placement of the APTOS thread, the thread was progressively surrounded by fibrous tissue and exhibited slow degradation (41.1% over 72 weeks). The prolonged tissue reaction guarantees stable and durable tread-lifting procedure results. The ability of Photic tissue repositions, nucleogenesis and neovascularization has a strong influence on skin texture, structure, color and body couture which is provided by APTOS threads composition and structure.*

Introduction

The desire to be attractive and to enhance or achieve physical beauty is as old as humankind itself [1]. As a result, aesthetic medicine has progressed in leaps and bounds due to constant advancements and innovations. Nowadays there is a huge demand for minimally invasive or non-invasive treatments, which do not require an extended recovery period and yield highly satisfactory results. Face-lifting with barbed threads is a leap for aesthetic medicine in the twenty-first century. The subcutaneous placement of barbed sutures lifts ptotic facial tissues. The concept of thread lifting and the first barbed threads were originally introduced by Dr. Marlen Sulamanidze in the late 1990s [2]. Since it is perceived as a safe and effective alternative to injectables and more invasive procedures such as facelifts for rejuvenating the face, thread lifting has attracted growing global interest. As a result, plastic surgeons, dermatologists, otolaryngologists, gynecologists, urologists and even nonsurgical specialists have employed a variety of threads that are based on Dr. Sulamanidze's original anti-ptosis suture (APTOS) threads [3].

The placement of threads subcutaneously generates a non-specific local immune response to the implanted foreign material which is known as the "foreign body reaction" [4]. This immune response lasts several months after the procedure and involves various cell types, including macrophages, lymphocytes, and mast cells. The granular products of these cells contribute to the formation of foreign body giant cells (multinucleated fused macrophages) and the formation of a dense connective tissue capsule around the implanted material. It is likely that this fibrotic response to implanted material plays an important buttressing role in the ability of barbed suspension threads to lift facial tissues and maintain their elevated position over the long term [5,6].

The histological changes that occur in the subcutaneous tissue after implanting barbed threads are currently poorly understood. Aptos Company conducted a study **to evaluate thread degradation time and the tissue reaction on thread material**. Rabbits underwent subcutaneous implantation of APTOS thread on one side of the back

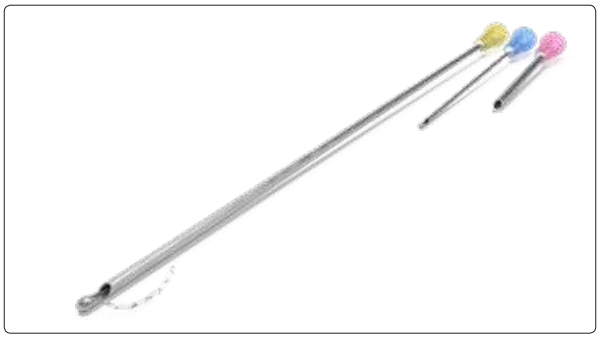
and a control thread on the other. The study objects were monitored for 72 weeks.

Materials and Methods

This study aimed to evaluate the degradation of and local tissue response to sutures and was conducted using a subcutaneous rabbit implant model according to Good Laboratory Practices (GLPs).

Suture Materials

Test Article 2 was the APTOS® Excellence Visage thread, Model ID: EV (Aptos LLC). It consists of 190 mm long poly(L-lactide-co-caprolactone) [P(LA/CL)] thread USP (Ø, 2/0) with multidirectional barbs that is preloaded in a 20G×150 mm non-traumatic rounded-tip cannula with a hole on the side (Figure 1). The **control suture material was 1 mm-diameter high density polyethylene (HDPE)** (provided by WuXiAppTec). All suture articles were sterile and ready to use. The needles associated with Test Article 2 were removed.



Animals

In total, **21 13-week-old female albino rabbits (*Oryctolagus cuniculus*, New Zealand White strain)** were used. All were from Charles River Laboratories, which is a previously approved vendor of commercial laboratory animals.

Procedure of Suture Material Implantation

The backs of 21 rabbits were shaved, after which they underwent aseptic surgery according to WuXiAppTec SOP: ILS-0003 for the subcutaneous bilateral placement of the test and control articles (approximately 5 cm in length). **The subcutaneous exposure route resembles the intended clinical exposure**. To ensure that the histopathology and degradation of the suture materials over time could be observed, the 21 rabbits were divided into seven groups of three. Tissue reaction was observed on 0, 4, 13, 26, 34, 52, 64, and 72 weeks after implantation, respectively. The time 0 Test Article tissue sections consisted of a small oval piece of clear acellular solid material and had a round to oval finely granular central region with a crescent shaped smooth area (barbs) on both sides of the central region. There was no tissue reaction to the time 0 Test Article material. The area of the time 0 Test Article material ranged from 0.0882 mm2 to 0.2016 mm2 with an average of 0.1534 mm2. In all groups, except the 26 weeks group, eight implants were placed in each rabbit. Specifically, four Test Article threads were implanted on the right side in the cranial-caudal direction and four control articles were implanted on the left side in the cranial-caudal direction (Figure 2). The 26 week animals received six dorsal subcutaneous implants only (three on each side). For implantation, dorsal subcutaneous implant pockets that were separated by approximately 1–1.5" of space were created and the implant materials were placed so that they lay as straight as possible parallel to each other. The incision sites were then surgically closed using standard surgical techniques (3-0 monocryl with or without suture glue). The entrance and exit of each implant tunnel were marked with a tattoo. After recovery, the rabbits were returned to their individual cages.

All Other Durations	
(n=3 animals per duration)	
CRANIAL	
Left	Right
Control	TA 2
Control	TA 2
Control	TA 2
Control	TA 2
CAUDAL	

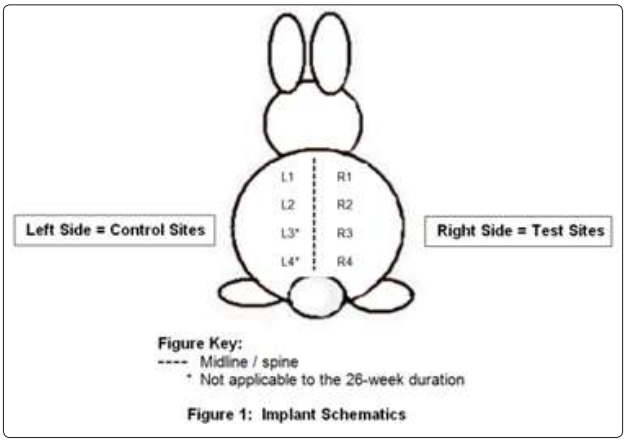


Figure 1: Implant Schematics

Tissue Collection and Preservation and Histological Analysis

All animals were treated according to WuXiAppTec SOP: ILS-0230 at the end of the scheduled observation period. The implant sites were subjected to gross observation and then harvested, fixed in formalin, processed by standard histopathology techniques, and embedded in paraffin. Two tissue sections from either side of the approximate midpoint of each sample were collected and stained with hematoxylin and eosin. The inflammatory cells and the connective tissues in the sections were evaluated using the keys shown in Tables 2 and 3, respectively. The Irritant Ranking Score (Test Article Group Average - the Control Article Group Average) was determined on the basis of Polymorphonuclear cells, Lymphocytes, Plasma cells, Eosinophils, Macrophages, Multinuclear Giant cells, Necrosis numbers (described as inflammation) and the degree of neovascularization and fibrosis (described as tissue response). The areas in the sections that were occupied by the implant materials were measured by outlining the implanted material or void area using Nikon Digital Sight software.

Results

Gross observations of the Test Article materials at 4, 13, and 26 weeks showed that one article was not observed (probably due to thread migration), one article appeared coiled in a round mass, and 10 articles displayed partial or slight bunching (±). The remainders were straight. After explant dissection, one of the Test Article materials exhibited only partial embedding in the surrounding tissue. Moreover, another article was difficult to feel and visualize due to overlying fat.

On 34 week 6 0ut of 12 Test Articles appeared to be fragmented. At 52 and 64 weeks, white tissue associated with Test Article of the

12 Test Article materials at 64 week, six out of 12 were very small and difficult to visualize. By 72 weeks, 10 of the 12 Test Article 2 materials were present as small fragments.

By contrast, gross observations showed that twelve control articles were not present because of the smooth and rod-shaped structure of the control article, movement/migration was commonly observed. At 4 weeks, all axillary lymph nodes had a black discoloration on control article side, and at 64 weeks, the right axillary lymph node were difficult to visualize; Therefore, tissue in the expected location was collected.

Histopathology

Inflammatory cells, neovascularization, and fibrosis over time

The Irritant Ranking Scores at the various timepoints were calculated. The scores showed that, compared with the control article at 4 and 26 weeks, Test Article was a non-irritant while, at 13, 34, 52, 64, and 72 weeks, it was a slight irritant.

Analysis of the local tissue reaction showed that Test Article and the control article caused the same tissue reaction, namely, chronic granulomatous inflammation and fibrosis with neovascularization. Specifically, at all timepoints, all Test Article implant sites contained minimal to moderate numbers of macrophages, minimal to mild numbers of polymorphonuclear cells, minimal numbers of lymphocytes and multinucleated giant cells, and minimal numbers of plasma cells. Eosinophils were rare (minimal numbers). Similar numbers of macrophages, polymorphonuclear cells, lymphocytes, multinucleated giant cells, plasma cells, and eosinophils were found around the control article at all implant sites at all timepoints (Table 1,2,3 and 4). However, compared with the control article, Test Article associated with greater numbers of inflammatory cells at 13 and 34–72 weeks (Figure 3). This difference is likely to be since Test Article is a degradable/absorbable material whereas the control article is non-degradable: the degradation of Test Article means that the tissue is constantly being stimulated, which provokes the influx of inflammatory cells into the tissues at the implant site.

Table 1. Histopathological Evaluation of connective tissue

		4 Week	13 Week	26 Week	34 Week	52 Week	64 Week	72 Week
macrophage	T	mild to moderate	mild to moderate	minimal to moderate	a mild to moderate	minimal to moderate	mild to moderate	mild to moderate
	C	mild to moderate	minimal to mild	minimal to mild	minimal to mild	minimal	minimal	minimal
polymorphonuclear	T	minimal to mild	Minimal to moderate	minimal	minimal	minimal to mild	minimal	minimal
	C	minimal to mild	Minimal to moderate	minimal	minimal	minimal to mild	minimal	minimal
lymphocytes	T	minimal	minimal to mild	minimal to mild	minimal to mild	minimal to mild	minimal	-
	C	minimal	minimal	minimal				
multinucleated giant cells	T	minimal	minimal	minimal	minimal to mild	minimal	minimal	minimal to mild
	C	minimal	minimal	minimal	minimal	minimal	minimal	minimal
plasma cells	T	minimal	minimal	minimal	minimal	minimal	minimal	minimal
	C	minimal	-	-	minimal	-	-	-
eosinophil	T	rare (minimal)	-	-	-	-	-	-
	C	-	-	-	-	-	-	-
maturing fibrous connective tissue (capsule)	T	minimal to mild	minimal to mild	minimal to mild	minimal	minimal to mild	minimal	minimal
	C	minimal to mild	minimal to mild	minimal	minimal	minimal	minimal	minimal
neovascularization	T	minimal to moderate	moderate	minimal to mild	minimal to mild	minimal to mild	minimal to ild	minimal to mild
	C	minimal to moderate	minimal to mild	minimal to mild	-	-	-	-
chronic granulomatous inflammation	T	+	+	+	+	+	+	+
	C	+	+	+	-	-	-	-

Table 2. Key for the histopathological evaluation of inflammatory cells

Inflammation	Score				
	Absent	Minimal	Mild	Moderate	Marked
Polymorphonuclear cells Lymphocytes Plasma cells Eosinophils Macrophages	0	Rare, 1–5/HPF	6–10/HPF	Heavy infiltrate	Packed
Multinucleated giant cells	0	Rare, 1–2/HPF	3–5/HPF	Heavy infiltrate	Sheets

HPF = high powered field (400×), averaged over the entire implant site.

Table 3: Key for the histopathological evaluation of connective tissue

Tissue response	Score				
	Absent	Minimal	Mild	Moderate	Marked
Neovascularization	Absent	Minimal capillary proliferation, (focal, 1–3 capillary buds), or small blood vessels (venules, and/or arterioles)	Groups of 4–7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis/fibrous capsule b	Fibrous capsule has not formed (i.e., cellular capsule is composed predominantly of inflammatory cells with little fibroplasia), or is too thin to measure	Narrow band (<100 µm mean thickness)	Moderately thick band (>100, up to 200 µm mean thickness)	Thick band (>200, up to 300 µm mean thickness)	Extensive band (>300 µm mean thickness)

Table 4: Derivation of the Group Avarage and Irritant Rank Score, and Interpretation of the Irritant Rank Score

	4 weeks	13 weeks	26 weeks	34 weeks	53 weeks	64 weeks	72 weeks
Sum Total Test article 2 Implant Scores	116	105	82	77	92	59	39
Number of Test article 2 Implant Sites Scored	12	11	12	9	12	10	6
Test article 2 Implant Site Group Average Score	9.7	9.5	6.8	8.6	7.7	5.9	6.5
Sum Total Control article Implant Scores	87	67	41	31	30	27	30
Number of Control article Implant Sites Scored	10	11	10	9	10	11	10
Control article Implant Site Group Average Score	8.7	6.1	4.1	3.4	3.0	2.5	3.0
Irritant Ranking Score	1.0	3.4	2.7	5.2	4.7	3.4	3.5
Interpretation of the Irritant Ranking Score	Non-irritant	Slight-irritant	Non-irritant	Slight-irritant	Slight-irritant	Slight-irritant	Slight-irritant

Key for Table 4:
The implant scores [I + TR] for each implant site scored are totaled. The Group Average = the Sum of the Total scores for that Group, divided by the number of implant sites, rounded to the nearest 10,h decimal point. **The Irritant Ranking Score is derived as follows:** Test article 2 Group Average Score - Control Article Group Average Score = The Irritant Ranking Score. **Interpretation of Irritant Ranking Score (negative values are treated as zero): Under the conditions of this study, as compared to the negative control score, the test sample was considered a:** • Non-irritant (0.0 up to 2.9) • Slight irritant (3.0 up to 8.9) • Moderate irritant (9.0 up to 15.0) • Severe irritant (> 15.0)

In terms of fibrosis and neovascularization, the Test Article implant sites contained fibrosis admixed with and internally lined by chronic granulomatous inflammation at all timepoints. In most, but not all, Test Article implant sites, the capsule was also generally admixed with minimal to moderate neovascularization. At all timepoints, the control article was surrounded by minimal mature fibrous connective tissue. In most, but not all, control article implant sites, the fibrotic tissue was admixed with minimal to mild neovascularization (Figure 3).

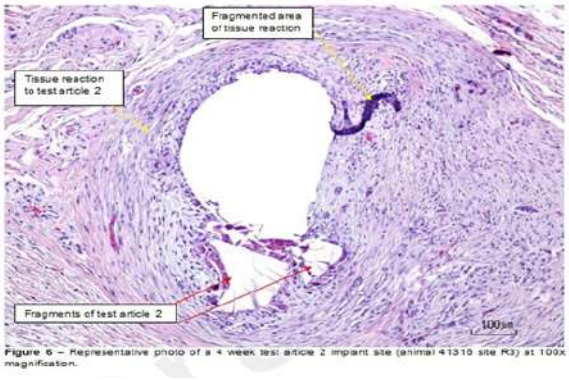


Figure 6 – Representative photo of a 4 week test article 2 implant site (animal 41319 site R3) at 100x magnification.

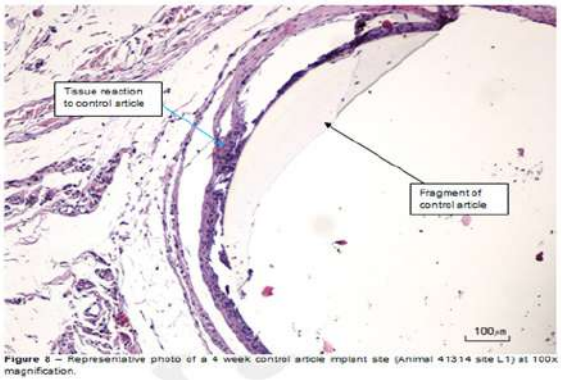


Figure 8 – Representative photo of a 4 week control article implant site (Animal 41314 site L1) at 100x magnification.

Figure 11 – Representative photo of a 13 week test article 2 implant site (animal 41318 site R1) at 100x magnification.

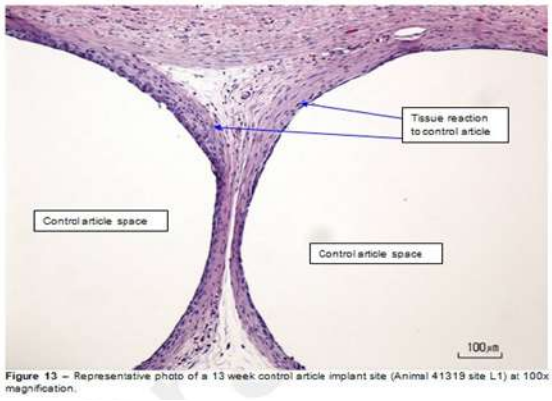


Figure 13 – Representative photo of a 13 week control article implant site (Animal 41319 site L1) at 100x magnification.

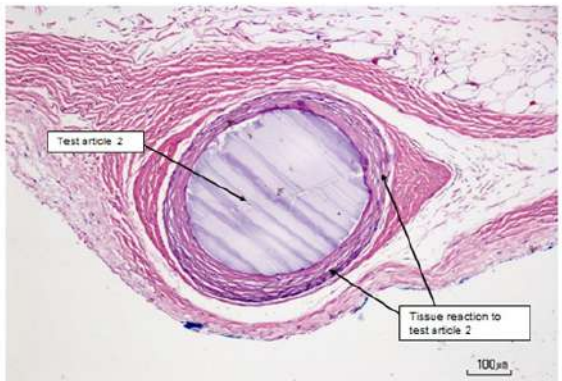


Figure 16 – Representative photo of a 26 week test article 2 implant site (animal 41325 site R3) at 100x magnification.

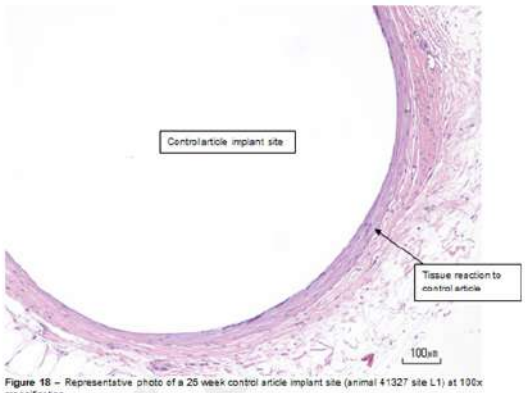


Figure 18 – Representative photo of a 26 week control article implant site (animal 41327 site L1) at 100x magnification.

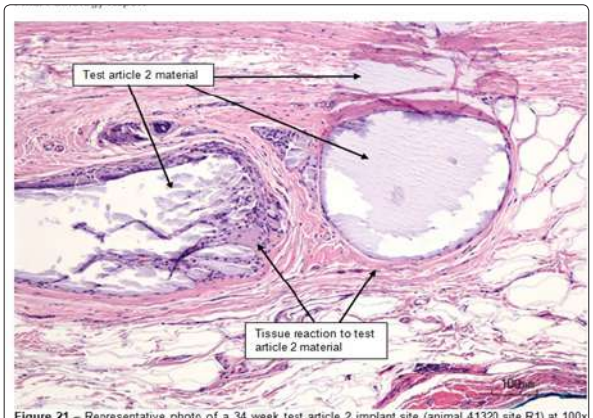


Figure 21 – Representative photo of a 34 week test article 2 implant site (animal 41320 site R1) at 100x magnification. The piece of test article 2 material above the cross-section was dragged out of the implant site during tissue processing.

Figure 23 – Representative photo of a 34 week control article implant site (animal 41328 site L1) at 100x magnification.

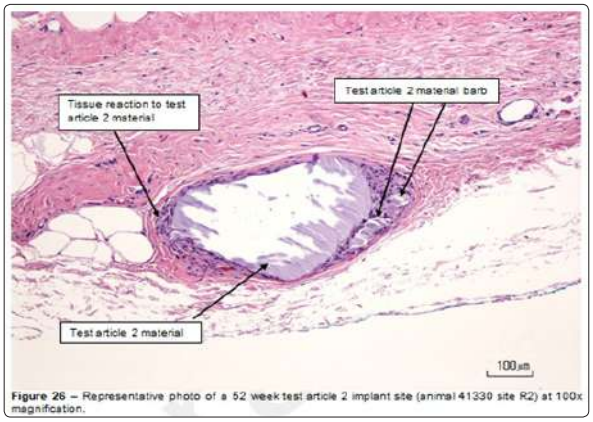


Figure 26 – Representative photo of a 52 week test article 2 implant site (animal 41330 site R2) at 100x magnification.

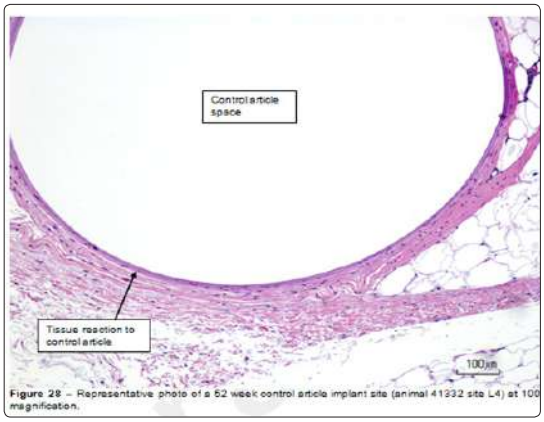


Figure 28 – Representative photo of a 52 week control article implant site (animal 41332 site L4) at 100x magnification.

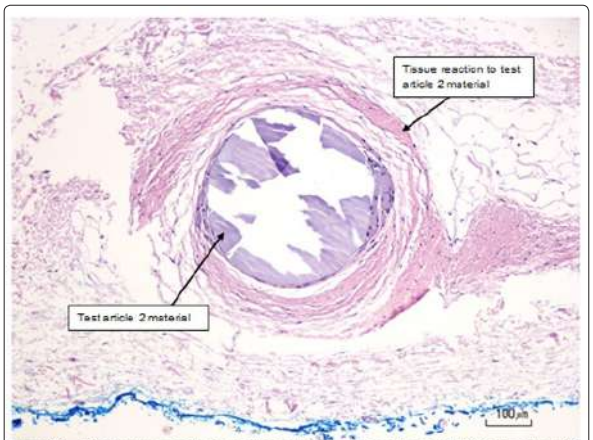


Figure 31 – Representative photo of a 64 week test article 2 implant site (animal 41334 site R1) at 100x magnification.

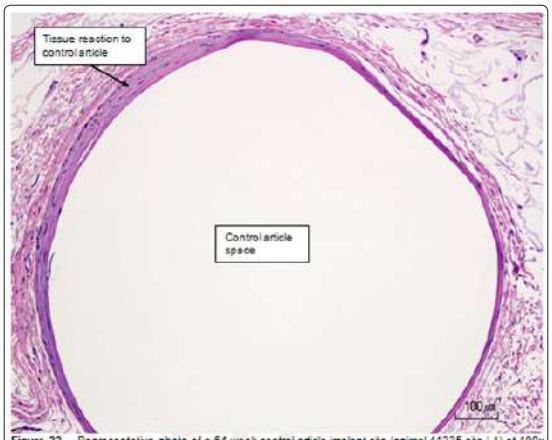


Figure 33 – Representative photo of a 64 week control article implant site (animal 41336 site L1) at 100x magnification.

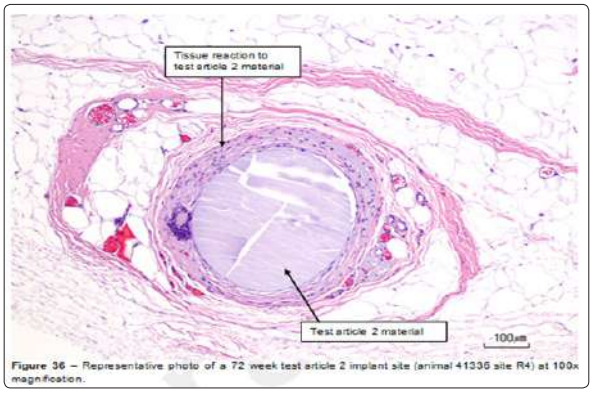


Figure 36 – Representative photo of a 72 week test article 2 implant site (animal 41336 site R4) at 100x magnification.

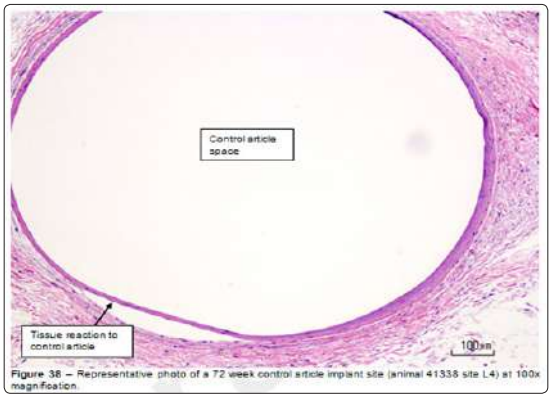


Figure 38 – Representative photo of a 72 week control article implant site (animal 41338 site L4) at 100x magnification.

Change in area of the implanted materials over time

At all timepoints, Test Article was generally largely intact. However, as shown by the average areas, it generally displayed increasing fracturing and infiltration by the tissue reaction over time. Specifically, compared with week 0, the average area of Test Article at week 4 rose by 0.0007%. Between weeks 4 and 13, and weeks 13 and 26, the average area decreased by 13.6% and 4.5%, respectively. Between weeks 26 and 34, the average area increased slightly by 4.7%. Between weeks 34 and 52, weeks 52 and 64, and 64 and 72, the average areas decreased by 15.0%, 15.0%, and 6.3%, respectively. Thus, overall, at 72 weeks, the average area of Test Article 2 had decreased by 41.1%. Despite the overall decline of the average area, however, the average area of Test Article did exhibit slight increase at 26 and 34 weeks of 0.1271 mm^2 0.1328 mm^2, respectively, relative to the preceding timepoint. It is likely that the average area at 26 and 34 weeks appeared to increase because one or more of the cross-sections of Test Article 2 taken at those timepoints were slightly oblique cuts of the implant rather than being exactly transverse (cross-section) cuts (Figure 4).

Figure 5 – Representative photo of a 4 week test article 2 implant site (animal 41316 site R3) at 40x magnification with area measurement of the test article 2 material.

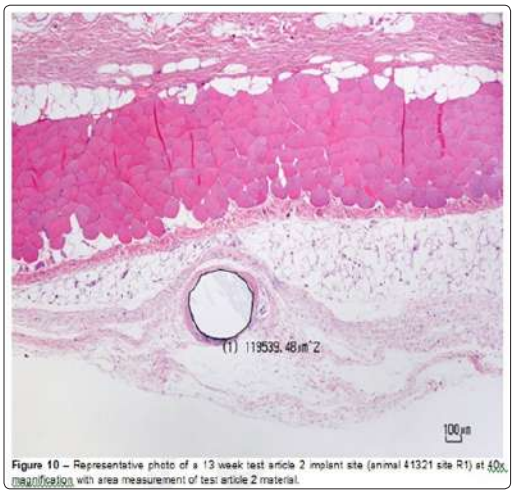


Figure 10 – Representative photo of a 13 week test article 2 implant site (animal 41321 site R1) at 40x magnification with area measurement of test article 2 material.

Figure 15 – Representative photo of a 26 week test article 2 implant site (animal 41325 site R2) at 40x magnification with area measurement of test article 2 material.

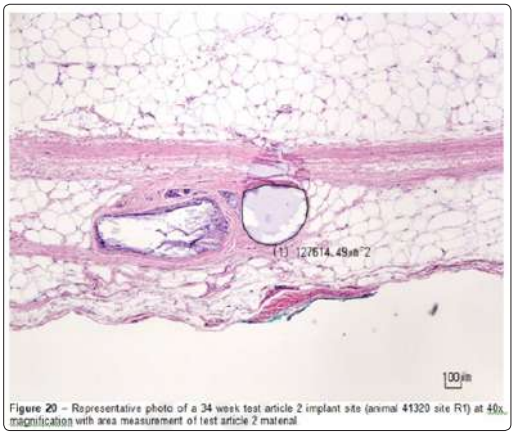


Figure 20 – Representative photo of a 34 week test article 2 implant site (animal 41320 site R1) at 40x magnification with area measurement of test article 2 material.

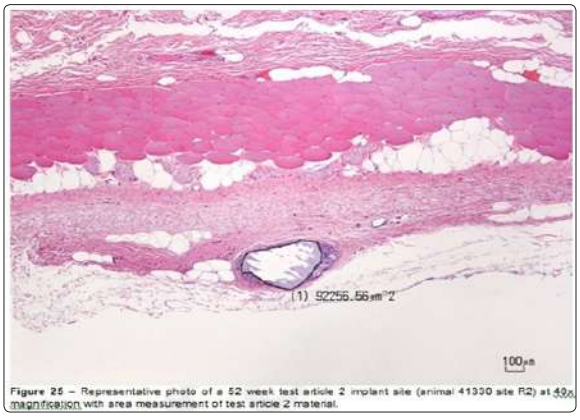


Figure 25 – Representative photo of a 52 week test article 2 implant site (animal 41330 site R2) at 40x magnification with area measurement of test article 2 material.

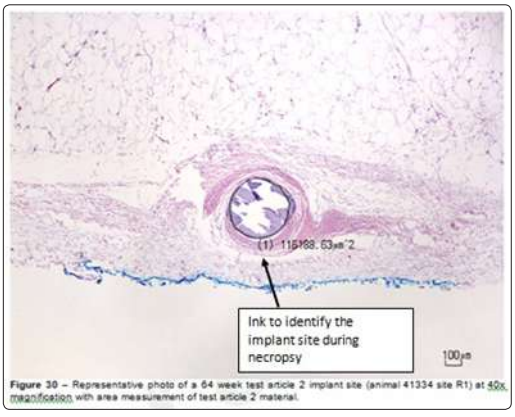


Figure 30 – Representative photo of a 64 week test article 2 implant site (animal 41334 site R1) at 40x magnification with area measurement of test article 2 material.

Figure 35 – Representative photo of a 72 week test article 2 implant site (animal 41336 site R4) at 40x magnification with area measurement of test article 2 material.

Figure 4: Measurement of the cross-sectional area of the Test Article 2 material between 4 and 72 weeks after implantation.

Notably, the average area of the control article also varied from 4 to 72 weeks even though it is a solid plastic material that should not change in area. It is also likely that this variation is due to oblique cutting of the control article implant sites (Figure 4).

Discussion

The thread lifting is used for facial rejuvenation, its popularity rises with growing demand on minimally invasive procedures. It is mini-invasive procedure that can be performed in 20 minutes under local anesthesia, providing aesthetically favorable results. The present study aimed first to evaluate the tissue response over 72 weeks of rabbits to two suture materials following dorsal subcutaneous implantation and second, to assess the *in vivo* degradation of the materials over time.

Implanted biomaterials provoke the recruitment and fusion of macrophages and the development of multinucleated foreign body giant cells. These cells are the dominant early responders to biomaterial implantation. They adhere to the biomaterial surface, and thereby promote fibrosis and, eventually, the development of a fibrous capsule around the implant material. The fibrosis also associates with intense neovascularization. All implanted biomaterials induce this cellular and tissue response, although the type of cells and tissue response do depend on the nature of the implanted biomaterial. In the present study, the histological analysis showed that the APTOS thread implantation induced a productive inflammation with macrophages and giant cells that was followed by the progressive growth of fibrous connective tissue around the threads and barbs and the formation of a homogeneous fibrous capsule around the thread. This fibrotic tissue and capsule formation is likely to ensure that the tissues that are lifted remain in place after the thread has degraded. **Therefore, it is possible to claim that the thread lifting effect is achieved and fortified by the cutaneous fibrotic reaction that appears along the length of the thread and that remains steady even when the thread has been completely absorbed**.

In terms of degradation, APTOS Excellence Visage revealed its biodegradable fractures. It was fractured and infiltrated during tissue reaction. Indeed, the average area of the Test Article implants decreased overall by 41% at the end of the 72 weeks observation period. The overall decrease in average area of control article implant sites between 4 (1.00 mm2) and 72 weeks (0.87 mm2) was approximately 0.13%. (Figure 3).

Comparison with the control implants showed that, while both Test Article and the control article caused chronic granulomatous inflammation and fibrosis with neovascularization, the Test Article 2 implants associated with higher numbers of inflammatory cells at 13 and 34–72 weeks than the control article implants (Figure 3). **This greater and more prolonged inflammation reaction can be caused by Test Article degradation which stimulates the body reaction**. By contrast, the non-degradable control article did not provide such prolonged immune stimulus. The size of the capsule changed along with the changes in the cell numbers and types.

Because it is very important to evaluate neocollagenesis during the scarring process, future studies should determine the types (I, III or any other) of collagen and elastin fibers present in the fibrous capsule.

Conclusion

After subcutaneous placement of the APTOS thread, the thread was progressively surrounded by fibrous tissue and exhibited slow degradation (41.1% over 72 weeks). The fibrous capsule is likely to provide the local soft tissues with internal support that holds them in an elevated position and prevents further ptosis. The degradation of the thread persistently stimulated inflammatory cell migration, and therefore increased fibrosis and neovascularization. This suggests that, **Test Article is likely to continue to degrade after 72 weeks and tissue stimulation will last until full degradation**. This prolonged tissue reaction guaranties stabile and durable thread lifting procedure results. Ability of Photic tissue reposition, neocollagenesis and neovascularization has strong influence on skin texture, structure, color and body couture which is provided by APTOS thread lifting procedures.

References

1. Rhodes G (2006) The Evolutionary Psychology of Facial Beauty. Annual Review of Psychology 57: 199-226. https://doi.org/10.1146/annurev.psych.57.102904.190208.
2. Sulamanidze M, Sulamanidze G (2009) APTOS Suture Lifting Methods: 10 Years of Experience. Clinics in Plastic Surgery 36: 281-306. https://doi.org/10.1016/j.cps.2008.12.003.
3. Sulamanidze M, Sulamanidze G, Sulamanidze C (2018) Elimination of Aesthetic Deformations of the Midface Area Our

Experience. Aesthetic Plastic Surgery. https://doi.org/10.1007/s00266-018-1112-3.
4. Cutright D, Hunsuck E (1971) Tissue reaction to the biodegradable polylactic acid structure. Oral Surgery, Oral Medicine, Oral Pathology 3: 134-139.
5. Sulamanidze M, Sulamanidze G (2008) Facial lifting with aptos methods. Journal of Cutaneous and Aesthetic Surgery. https://doi.org/10.4103/0974-2077.41149.
6. Luis Manuel Orozco-Castellanos, Angel Marcos-Fernández, Antonio Martínez-Richa (2011) Hydrolytic degradation of poly (ε -caprolactone) with different end groups and poly (ε -caprolactone-co- γ -butyrolactone). Characterization and kinetics of hydrocortisone delivery . Polymers for Advanced Technologies 22: 430-436. https://doi.org/10.1002/pat.1531.